

**RISK FACTORS AND SEROPREVALENCE OF MYCOPLASMA SYNOVIAE INFECTION
IN BROILER BREEDER FARMS IN MAZANDARAN PROVINCE, NORTH IRAN**

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Mycoplasma Synoviae (MS) is a considerable and economically crucial pathogen for avian species. This study was aimed to determine the risk factors (age, size of flock, season, and strain) and seroprevalence of M. synoviae in broiler breeder farms in Mazandaran province, north of Iran. The study was conducted from May 2002 to October 2008. MS was confirmed by Rapid Serum Plate Agglutination (SPA) and Enzyme Linked Immunosorbent Assay (ELISA) tests. Broiler breeder farms (n=315) were followed for this study and three samples for every thousand were obtained randomly. Sera that were SPA positive were analyzed for antibodies against MS using a commercially available ELISA antibody test kit. The highest (41.2%) and lowest (0%) prevalence of MS infection was found in 2003 and 2008, respectively. Results showed that the prevalence of MS infection increased with the increase of age. No relation between a special breed with M. synoviae status could be found. Seasonal variations of prevalence with MS infection were observed in the present study. The population of the flocks did not influence Mycoplasma Synoviae prevalence. The results showed that occurrence of M. synoviae has a significant relationship with the age and sampling district.

Key words: broiler breeder, Mazandaran, Mycoplasma synoviae, risk factors, seroprevalence

INTRODUCTION

Mycoplasmas are very small prokaryotes devoid of cell walls, bounded by a plasma membrane only (Kleven, 2008). *Mycoplasma synoviae* (MS) is one of the most important pathogenic *Mycoplasmas* for chickens. Less frequently, MS becomes systemic and results in infectious synovitis, an acute to chronic infectious disease of chickens and turkeys, involving primarily the synovial membranes of joints and tendon sheaths, and bursitis (Kleven and Ferguson-Noeln, 2008). As vertical transmission plays a major role in spreading of MS, in chickens the most effective method of control is regular flock monitoring and elimination of positive breeder flocks. Reliable and rapid diagnosis is needed to prevent dissemination of infection (Kleven and Ferguson-Noeln, 2008; Lockaby et

al., 1998). Provisional diagnosis and monitoring of *M. synoviae* infection is usually made using serological assays. The most used serological tests are serum plate agglutination (SPA), hemagglutination inhibition (HI), and enzyme linked immunosorbent assay (ELISA) (Kleven, 1998). SPA reactors must generally be confirmed by the HI or ELISA tests. In general the ELISA test is more sensitive than the HI test and more specific than the SPA test (Kempf *et al.*, 1994; Kempf and Gesbert, 1998). Culturing of MS could be costly, time-consuming and inconclusive (Ewing *et al.*, 1996). In Iran, SPA is used for flocks monitoring and as a screening test. SPA is used as the screening test because it's rapid, has high sensitivity, and low specificity, as well as being inexpensive (Kleven, 1998). ELISA has been proved to have good sensitivity and more specificity compared to SPA. Some factors such as: age, size of flock, location, etc., may affect the severity of this disease. The aim of the present study was to determine some of the risk factors (age, size of flock, season and strain) and seroprevalence of *Mycoplasma synoviae* (MS) in broiler breeders in Mazandaran province, north of Iran.

MATERIALS AND METHODS

Samples

The study was conducted at the commercial farms of Mazandaran province, from May 2002 to October 2008. 315 broiler breeder farms were followed for this study and three samples for every thousand were obtained randomly. The sampling was done according to the guideline approved by the Iranian veterinary organization. Use of birds in this study was approved by the local institutional Animal Ethical Committee.

Blood collection and serum preparation

Blood samples were aseptically collected from the wing vein using 5-mL sterile disposable syringes and needles. Blood was allowed to clot in the syringe and was kept for about 1 hour at room temperature. After this, serum of each sample was separated, centrifuged, and transferred to sterile microtubes kept at -20°C until analysed.

Serum Plate Agglutination (SPA) Test

All serum samples were inactivated by heating at 56°C for 30 minutes to destroy nonspecific inhibitory substances and tested for MS by SPA, using a commercial antigen for the diagnosis of *Mycoplasma synoviae* by serum plate agglutination, according to the manufacturer's instructions. In short, 0.02 mL of the serum to be tested was mixed with 0.02 mL of the commercial antigen (1:1) on a glass plate. After that, the plate was placed under a light source, and samples that showed agglutination (presence of clots) were considered positive. Positive sera were diluted 1:5 and 1:10 with 0.5 M phosphate-buffered saline (PBS), pH 7.2. Both dilutions were tested again by SPA as described above. Sera were considered positive when clots were observed in dilutions up to 1:10.

Enzyme-Linked Immunosorbent Assay (ELISA)

Sera that were SPA positive were analyzed for antibodies against MS using a commercially available ELISA antibody test kit according to the manufacturer's instructions. Briefly, samples were diluted five-hundredfold (1:500) with the diluent, and 0.1 mL of each sample was dispensed in a well of a plate previously coated with MS antigen. Plates were incubated for about 30 minutes at room temperature. After that, plates were washed with deionized water, and 0.1 mL of the conjugate was placed in each well (Goat antichickens: horseradish peroxidase conjugate HRPO). Plates were incubated for about 30 minutes and washed again. Finally, 0.1 mL of the substrate solution (tetramethylbenzidine or TMB) was dispensed into each well and incubated for 15 minutes at room temperature. The reaction was blocked with 0.1 mL of stop solution. Absorbance was measured at 650 nm. Results were expressed as serum-to-positive ratios (S/P ratios) relative to a standard positive control. Serum samples, with S/P ratios greater than 0.5 (titers greater than 1 076) were considered positive.

Statistical analysis

Results were analyzed using SPSS (Chi square test and Pearson correlation).

RESULTS

The choice of the SPA test to study the seroprevalence of *M. synoviae* was based on previous research in which the specificity and sensitivity of this laboratory test was compared with that of culture, polymerase chain reaction (PCR) and various commercial enzyme-linked immunosorbent assay kits. The SPA test was found to perform equally as well as the tested enzyme-linked immunosorbent assay kits (Ferberwee *et al.*, 2005). Sera samples were collected during seven years. The highest (41.2%) and lowest (0%) Prevalence of MS infection was found in 2003 and 2008 respectively (Table 1). The prevalence was highest (39.6%) in winter and lowest (30.6%) in summer. Ross, Cobb, Arian, Hubbard and Arbor Acres had 40%, 44%, 32%, 27% and 45% respectively. The prevalence of MS was recorded highest at above 60 wk (43.1%), whereas the prevalence was lowest (12.7%) at 10-20 weeks of age ($p < 0.05$). Farms were separated in two different zones (foothills with less humidity compared with coastal area). The prevalence of Mycoplasmosis in foothills was significantly ($p < 0.05$) higher (36.9%) than in the coastal area (30.1%). No significant difference was seen in flocks up to 30 000 population (37.8%), 30 000-40 000 (46.8%) and over 40 000 (51.3%).

Table 1. Relationships between *Mycoplasma synoviae* infection and various risk factors (Mazandaran, Iran)

Risk factor	Level of risk factor	Positive flocks (%)	Significance of difference (p value)
Age (weeks)	10-20	12.7	0.000
	20-30	23.6	
	30-40	30.6	
	40-50	32.8	
	50-60	40.5	
	Above 60	43.1	
Season	Spring	35.1	0.123
	Summer	30.6	
	Autumn	32.5	
	Winter	39.6	
Breed	Ross	40	0.000
	Cobb	44	
	Arian	32	
	Hubbard	27	
	Arbor acres	45	
Flock size	Up to 30 000	37.8	0.112
	3 000-40 000	46.8	
	Above 40 000	51.3	
Year	2002	37.1	0.138
	2003	41.2	
	2004	32.4	
	2005	31.3	
	2006	36	
	2007	24.8	
	2008	0	
Zone	Coastal area	30.1	0.027
	Foothills	36.9	

DISCUSSION

M. synoviae can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996). Results showed that the prevalence of MS infection increased with the increasing age. Culling of *M. synoviae*-positive flocks reduces the risk of vertical transmission of this mycoplasma to the offspring, which is reflected in the low seroprevalence of *M. synoviae* in rearing breeder stock (12.7%). Similar report was demonstrated in another study that reported lowest MS infection (6%) at rearing age (Feberwee *et al.*, 2008). As the risk of vertical transmission is minimized by culling *M. synoviae*-infected flocks, horizontal transmission is expected to be the most important transmission route for infected rearing breeder

flocks. Most *M. synoviae*-seropositive submissions originated from breeder flocks above 60 weeks of age (43.1%). This finding is in agreement with data of other research groups. Feberwee *et al.* (2008) reported *M. synoviae*-seropositive birds in layer type breeder flocks ≥ 51 weeks of age (60% positive flocks). In a prevalence study which was based on the detection of *M. synoviae* antibodies in eggs, a prevalence of 78.6% in commercial layer flocks in East England was reported by Hagan *et al.* (2004). In another study, a *M. synoviae* prevalence of 87% was found in commercial layer flocks in Southern California (Mohammed *et al.*, 1986). The infection was associated with older flocks that had molted or were frequently medicated. The high prevalence and persistence of *M. synoviae* infections in layer stock can be explained by the frequent occurrence of multiple age housing and lower biosecurity standards in this sector (Kleven and Ferguson-Noeln, 2008; Stipkovits and Kempf, 1996).

In this study the lowest prevalence (0%) of MS infection (found in 2008) might be due to sampling time. In 2008, sampling was done just in the first half of the year and the seroprevalence in this period was zero, in this year many broiler breeder flocks in Mazandaran province were vaccinated against *Mycoplasma Synoviae* (MS-H), so we had to stop sampling, because serologic tests would not be accurate and molecular testing was needed.

No relation between a special breed with *M. synoviae* status could be found which was in agreement with the result of Dufour-Gesbert *et al.* (2006). The prevalence of MS was significantly higher at the age above 60 weeks. However, season and size of flock do not have an impressive effect on the appearance of *Mycoplasma synoviae* infection, but this problem is worse in large flocks. For this reason, lower incidence was observed in flocks up to 30 000 birds in each farm. Serological investigation showed the highest infection rate (51.3%) in large scale flocks (>40 000 birds) in comparison (37.8%) to small (up to 30 000 birds) flocks. Similar report was described in another study (Dufour-Gesbert *et al.*, 2006).

In the present study seasonal variation for prevalence of MS infection was observed. The prevalence was highest (39.6%) in the winter and lowest (30.6%) in the summer which was in agreement with the results of other researchers (Heleili *et al.*, 2011; Hossain *et al.*, 2010; Sarkar *et al.*, 2005; Sikder *et al.*, 2005). It might be due to the influence of cold weather. The statistical analysis by one way ANOVA method (F-test) showed no significant variation between the prevalence of seasons.

Regional variations of prevalence of *M. synoviae* were observed in the present study. The prevalence of Mycoplasmosis in the foothills was significantly ($p < 0.05$) higher (36.9%) than in the coastal area (30.1%). Density of flocks was higher in the foothills than in the costal area, probably this factor influenced the prevalence of MS in these regions. As in Hagan *et al.* (2004) study, infection was more frequent on sites with several houses. This risk factor was also observed for other diseases or infections (Refregier-Petton *et al.*, 2001; Skov *et al.*, 1999). Indeed Goodwin (1985), described that, for another mycoplasma disease, i.e. enzootic pneumonia in pigs, the geographical position of an uninfected herd in relation to infected herds was the dominant factor for outbreak. This study confirmed the high prevalence of *M. synoviae* infection in broiler breeder farms in

Mazandaran province. Furthermore, the results show that the occurrence of *M. synoviae* has a significant relationship with the age of flocks and zone of sampling.

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FAKTORI RIZIKA I SEROPREVALENCIA *MYCOPLASMA SYNOVIAE* INFEKCIJE NA FARMAMA BROJLERA U IRANSKOJ SEVERNOJ PROVINCIJI MAZANDARAN

SEIFI S

SADRŽAJ

Mycoplasma synoviae (MS) je važan i ekonomski značajan patogen za ptice. Ova studija je sprovedena sa ciljem da se utvrde faktori rizika (uzrast, veličina jata, lokalitet i soj) na seroprevalencu infekcija sa *Mycoplasma synoviae* na farmama brojlera u iranskoj severnoj provinciji Mazandaran. Ispitivanja su izvedena od maja 2002. do oktobra 2008. godine, brzom serumskom aglutinacijom (SPA) i ELISA metodom. Ukupno je analizirano 315 jata, a uzimana su po tri uzorka krvi na svakih 1000 brojlera metodom slučajnog izbora. Uzorci seruma, koji su bili pozitivni u SPA testu, dodatno su ispitivani komercijalnim ELISA testom na prisustvo antitela protiv MS. Prevalenca MS infekcija se povećavala sa uzrastom ptica ali nije zavisila od soja brojlera i veličine jata. Dokazane su i sezonske varijacije u pojavljivanju MS infekcije brojlera.

